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5 METHODS FOR PREVENTING CROSS-CONTAMINATION IN SOLID SUPPORT-BASED ASSAYS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention pertains to the field of improving the reliability and sensitivity of various assay formats in which a solid support is used. The invention provides methods of reducing cross-contamination of reagents used in, for example, nucleic acid hybridization assays and immunoassays.

Background

The ability to detect a specific molecule in an impure sample is a cornerstone of many modern diagnostic and research methods. For example, prior to the advent of modern techniques, diagnosis of an infectious disease often required one to isolate the pathogenic organism from a biological sample, enrich the organism, and finally identify the organism. Because many infectious diseases require quick intervention in order for a successful treatment, the time and labor required for culturing and identifying the organism limited the usefulness of these methods. Moreover, these methods were useful only for samples that contain a viable pathogen. For diseases that are not caused by infectious organisms, for example, such methods were not useful.

More recent assays rely on detection of a molecule associated with a disease condition, rather than on detection of a pathogen itself. Often, these assays involve detecting a component of a pathogen in a biological sample. Immunoassays have long been used to detect specific molecules for which an antibody is available (*see, e.g.*, US Patent No. 5,656,448). Detection of nucleic acids associated with a pathogen provides another avenue by which the clinician can detect a pathogen or other disease-related condition (*see, e.g.*, US Patent No. 4,563,419). To facilitate detection of a molecule of interest in a crude sample, solid-phase assays have been developed. For example, one can immobilize on a solid support

an antibody that binds to a molecule that is associated with the pathogen or disease condition. The solid support is then contacted with the sample, and the molecule of interest, if present in the sample, binds to the solid support. The sample is then removed and the molecule of interest is detected.

5 Although assays such as those described above constitute a great advance over previously available diagnostic and research assays, problems with the assays persist. For example, an assay can yield a negative result even though the sample actually contains the target analyte of interest. In other cases, one or more of the assay components can precipitate out of solution, thus rendering the assay result unreliable. Thus, a need exists for methods of obtaining more sensitive and reliable results from diagnostic and other assays. The present invention fulfills this and other needs.

SUMMARY OF THE INVENTION

10 The invention provides methods of reducing cross-contamination of reagents in various types of assays and other experiments that involve transfer of a solid support from one reagent to another. For example, in one embodiment the invention is used where an assay involves contacting a solid support with a first reagent solution, removing the solid support from contact with the first reagent solution, and contacting the solid support with a second reagent solution. To reduce cross-contamination of the second reagent solution by the first reagent solution, the solid support is coated with a non-stick material prior to contacting the solid support with a first reagent solution. The solid support can be contacted with one or more intermediate reagent solutions (*e.g.*, a wash solution) prior to contacting the solid support with the second reagent solution.

15 The invention also provides methods of detecting a target analyte in a test sample by contacting the test sample with a solid support that is coated with a non-stick coating material prior to contacting the sample. Attached to the support is typically a capture reagent that binds to the target analyte. The solid support is then contacted with a signal reagent which binds to the target analyte, the presence or absence of the target analyte is determined by detecting the presence of signal reagent immobilized on the solid support.

20 In another embodiment, the invention provides an apparatus for detecting a target analyte. The apparatus includes a solid support to which is attached capture reagent

that binds to the target analyte. The solid support is coated with a non-stick coating material, which reduces or eliminates cross-contamination of reagent solutions when the solid support is moved from one reagent solution to another.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows results obtained in a nucleic acid sandwich hybridization assay using uncoated prongs or prongs that had been coated with GelSlick™. The absorbance is plotted versus the amount of streptavidin used to coat the prongs after the non-stick coating was applied.

Figure 2A and Figure 2B show the results obtained in a nucleic acid sandwich hybridization assay in which GelSlick™-coated prongs were used. The results in Figure 2A were obtained using adjusted volumes of hybridization and assay wash solution, while those of Figure 2B were obtained using standardized assay reagent volumes of 200 µl.

DETAILED DESCRIPTION

Many assay formats, including immunoassays and nucleic acid hybridization assays, can suffer from artifactual results such as false negatives and interference with signal detection. The present invention is based on the discovery that these and other artifacts are often the result of reagents involved in signal generation becoming contaminated with reagents from an earlier step of the assay. For example, it was found that in an assay that uses an enzyme-linked detection agent, the introduction of agents such as detergents and chaotropic agents from a cell lysate to the solution that contains the substrate for the enzyme can result in incorrect assay results. These contaminants can interfere with the assay in a number of ways. For example, the introduction of a detergent or other denaturant into the enzyme substrate solution can cause precipitation of the enzyme substrate or inactivation of the enzyme. The inventors discovered that solid supports used in the assays could carry non-specifically bound contaminants from one reagent solution to another, thus causing these adverse effects. Accordingly, the invention provides methods for reducing or eliminating artifactual results that are observed in many types of assays.

Assay Formats

The methods of the invention are useful in assay formats that involve the use

of a solid support; in particular, assays in which the solid support comes into contact with

more than one solution used in the assay. For example, in some assays a target analyte present in a test sample becomes bound, either directly or indirectly, to a solid support which is placed in the test sample. The solid support to which the target analyte is bound is then transferred sequentially to one or more other solutions that contain, for example, a detection system, a wash solution, and the like. Alternatively, the test sample is removed from the solid support and a different solution is placed in contact with the solid support. At each step, the solid support comes into contact with a different set of reagents. As described in more detail below, cross-contamination of one reagent with another can interfere with the assay.

One example of an assay format for which the methods of the invention are applicable is a sandwich assay. Sandwich assays are typically performed by placing a test sample in contact with a solid support. Immobilized on the solid support are a plurality of capture moieties to which the target analyte binds, if present in the sample. After incubation for a sufficient time to bind a substantial portion of the immobilized target analyte, the solid support is generally washed to remove unbound reagents. A detection reagent that is specific for the target analyte is then brought into contact with the target analyte, which is bound to the solid support. The solid support to which is bound the target analyte and the detection reagent is then contacted with a signal development solution that contains reagents necessary for detection of the detection reagent. The presence or absence of a detectable signal is then determined by methods appropriate for the particular label employed. For example, in the case of an enzyme used as a detectable label, a substrate for the enzyme that turns a visible color upon action of the enzyme is placed in contact with the bound detection moiety. A visible color will then be observed in proportion to the amount of the specific antigen in the sample.

An illustrative example of a sandwich assay for which the non-stick coated solid supports of the invention are useful is a nucleic acid hybridization assay. These assays typically involve attaching a capture probe, which is an oligonucleotide that is complementary to a portion of a target nucleic acid, to a solid support. The test sample is contacted with the capture probe (before, after, or simultaneously with attachment of the capture probe to the solid support) under conditions suitable for the target nucleic acid to hybridize to the capture probe. The solid support is then contacted with a signal probe, which

is generally a labeled oligonucleotide that is complementary to a portion of the target nucleic acid other than that portion to which the capture probe binds. After incubation under suitable hybridization conditions, the presence of label is then detected, typically by contacting the solid support with a signal development reagent.

The nucleic acids detected in these assays can be DNA, RNA, or heterohybrids. In some embodiments, the target nucleic acid is amplified prior to detection to increase the sensitivity of the assay (see, e.g., US Patent No. 5,374,524). Suitable amplification methods include, for example, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), strand displacement amplification (SDA), Q β -replicase amplification, nucleic acid sequence-based amplification (NASBA), transcription mediated amplification, and the like. Examples of techniques sufficient to direct persons of skill through such *in vitro* amplification methods, are found in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Vols. 1-3, Cold Spring Harbor Laboratory; Berger and Kimmel (1987) *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning Techniques*, San Diego: Academic Press, Inc.; and Ausubel *et al.* (1987; 1998 Suppl.) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York), as well as in Mullis *et al.* (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis), Fahy *et al.* (1991) in *PCR Methods and Applications*, Cold Spring Harbor Laboratory Press, pp. 25-33. Reverse transcriptase PCR is described in, for example, Kawasaki (1990) in *PCR Protocols: A Guide to Methods and Applications*, M.A. Innis *et al.*, eds., pp. 21-27.

Another illustrative example of an assay for which the methods of the invention are useful is an immunoassay. In a solid support-based sandwich immunoassay, for example, a capture reagent is attached to a solid support. The test sample is placed in contact with the capture reagent (before, after, or simultaneously with attachment of the capture probe to the solid support) under conditions suitable for the target analyte to bind to the capture reagent. The solid support is then contacted with a labeled signal reagent that binds to the target analyte, if present on the solid support.

Causes of Assay Artifacts

A common feature of the assay formats described herein is that a solid support comes into contact with more than one solution during the course of the assay. Thus, failure to remove all reagents from the solid support before contacting the solid support with another assay solution can result in cross-contamination. Cross-contamination is particularly problematic if a reagent that is carried over can interfere with a subsequent step of the assay.

Reagents that can interfere with subsequent assay steps, if cross-contamination is not prevented, include many compounds that are used in sample preparation. For example, cell lysates that are used as the sample for immunoassays and other types of assays often contain a detergent. Detergents that are commonly used for sample preparation include non-ionic detergents such as epoxy-fatty acid esters (e.g., Tween 20 (polyoxyethylene-sorbitan monolaurate), Tween 80 (polyoxyethylene-sorbitan monooleate), Triton (alkyl-polyether-alcohol mixtures), Brij 35 (polyoxyethylene lauryl ether), Nonidet P-40 (polyoxyethylene octylphenol ether), Lubrol PX (polyethylene oxide-alkyl ether adduct), Berol EMU 043 (C16, C18 fatty alcohol with 10 oxyethylene units) and the like. Anionic detergents such as, for example, sodium dodecyl sulfate (SDS) are also used. Any of these detergents, if carried from the sample to another solution used in the assay, can cause assay failure.

Other types of denaturant that can cause adverse effects on an assay are chaotropic agents. Chaotropic agents are often used in preparation of samples for use in, for example, nucleic acid detection assays. The chaotropic agent stabilizes nucleic acids by inhibiting nuclease activity. Furthermore, the chaotropic agent allows sensitive and stringent hybridization of short oligonucleotide probes to RNA at room temperature (Van Ness and Chen (1991) *Nucl. Acids Res.* 19: 5143-5151). Suitable chaotropic agents include guanidinium chloride, guanidinium thiocyanate, sodium thiocyanate, lithium tetrachloroacetate, sodium perchlorate, rubidium tetrachloroacetate, potassium iodide, urea, and cesium trifluoroacetate, among others. Typically, the chaotropic agent will be present in a sample at a final concentration of about 1-3 M. These compounds, if bound to the solid support, can resolubilize during a later step and lead to artifactual results in the assay.

Enzyme-based detection systems, which are described in more detail below, provide an example of some types of artifactual results that can result from cross-contamination. Carryover of substances such as denaturants, detergents, and chaotropic

agents to, for example, the solution that contains the substrate for the enzyme can cause artifactual results in at least two ways. First, the contaminant can cause precipitation of the substrate, thus reducing the amount of signal generated and/or causing an increase in absorbance due to blocking of light caused by the precipitated material. Second, the 5 contaminant can cause inactivation of the enzyme. Again, this can cause a reduction of signal from the sample.

Previously known assay formats often attempted to prevent cross-contamination by washing the solid support in between contact with the different reagents. However, the inclusion of washing steps has not eliminated artifactual results. The inventors have discovered that washing does not necessarily eliminate cross-contamination due to carryover by the solid support. Reagents present in one assay solution can precipitate onto a solid support and subsequently become resolubilized in a solution used in a later assay step. Accordingly, the present invention provides methods of reducing or eliminating this cross-contamination due to solid support carryover.

Prevention of Cross-Contamination

The invention provides methods of reducing or eliminating cross-contamination in assays due to solid support carryover. The methods involve coating the solid support with an agent that prevents non-specific binding to the support of reagents that can interfere with subsequent steps of an assay. Coating the supports with an appropriate 20 non-stick coating agent minimizes carryover of such contaminants to a later reaction mixture.

Prior to coating with a non-stick reagent, solid supports can be pre-treated with, for example, a cleaning and/or sterilizing solution such as, for example, ethanol, methanol, propanol, and the like. The supports are generally dried prior to the coating step.

To coat the solid supports with silanes, the solid support is contacted with a 25 solution that contains the non-stick reagent. Contacting can be accomplished, for example, by dipping, pouring, spreading, spraying, soaking, or any other method that can coat at least the portion of the solid support that will come into contact with assay reagents. The solid support is kept in contact with the non-stick reagent for a sufficient time for the support to 30 become coated. For example, in the case of a silane or GelSlick™ the coating time is typically between about one second and about twenty minutes, more preferably between

about one minute and about ten minutes. Automated plate washers provide a particularly convenient way in which one can carry out the plating steps.

The coating material can be any compound that can prevent a potential cross-contaminant from sticking to the solid support. Several types of non-stick coatings are known to those of skill in the art. Among these are silanes such as, for example, dimethylchlorosilane, and the like. The concentration of silanizing agent in the solutions can depend on the particular solid support. For some supports, the limiting factor for maximum silane concentration is the stability of the support in the silane solution. For example, a suitable coating solution for polystyrene supports will contain between about 0.01% and about 50% silanizing agent (v/v), more preferably between about 0.1% and about 5%, in a solvent such as water or ethanol.

One example of an alternative coating agent is GelSlick™ (FMC Corporation, Chicago IL), which is a proprietary non-toxic alternative to silane-based coatings. Coating with GelSlick™ can be accomplished by contacting the solid support with undiluted GelSlick™; alternatively the coating solution can contain GelSlick™ diluted in an organic and/or aqueous solvent. In a presently preferred embodiment, the concentration of GelSlick™ in the coating solution is between about 40-60% in 40-60% ethanol. Another presently preferred coating solution contains about 2% GelSlick™ in water. Aqueous solutions of GelSlick™ are preferably emulsified by sonication, thus resulting in more efficient coating of a solid support.

As discussed below, a capture reagent that is specific for a particular target analyte is often attached to a solid support. Significantly, one can coat the solid support with the non-stick material according to the methods of the invention either before or after attaching a capture reagent to the support. The non-stick material does not interfere with the ability of capture reagents to bind to their respective target analytes. This is particularly advantageous for commercial-scale preparation of solid supports for use in assays.

The methods of the invention are particularly useful in assays that are carried out using an automated analyzer. Typically, each well in a row of a microtiter plate contains one either the sample or one of the reagents to be used in the assay. For example, a first row of wells might contain the capture reagent, the second row might contain the test samples, the signal reagent might be in the third row, a wash solution in the fourth row, and the

substrate for the enzyme linked to the signal reagent in the fifth row. The automated analyzer moves a solid support, such as a strip of polystyrene prongs or dipsticks, from one row of wells to the next. The prongs are thus placed sequentially in each of the assay reagents for an appropriate time for the particular binding or other reaction to occur. The amount of signal for each well is then determined. Suitable automated analyzers are known to those of skill in the art. Coating of the prongs with a non-stick coating according to the invention can reduce or eliminate dropouts and/or substrate precipitation caused by carryover from one well to another.

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Solid Supports

The methods of the invention are useful for a wide variety of solid supports. These include, but are not limited to, dipstick-type devices, immunochromatographic test strips and radial partition immunoassay devices, and flow-through devices. The solid support can be, for example, a container in which the assay steps are performed (e.g., a well of a microtiter plate, a test tube, a centrifuge tube, and the like), or can be an entity that is moved from one reaction to another (e.g., a dipstick or prong). For example, in some types of assays for which the methods of the invention are applicable, a sample is placed into contact with a solid support to which a target analyte of interest binds. The sample solution is then removed from contact with the solid support, after which a second solution is placed into contact with the solid support. The solid support can be, for example, a container into which the reagent solutions are placed. Alternatively, the reagent solutions can be in different containers and the solid support moved from one container to the next. In either case, the solid support is often contacted with an intermediate solution, e.g., a wash solution or a solution containing other assay components, in between contacting the first solution and the second solution.

The invention is applicable to solid supports that are made of many different materials, including, for example, glasses, plastics, polymers, metals, metalloids, ceramics, organics, and the like. Specific examples include, but are not limited to, polystyrene prongs, silicon chips, microtiter plates, glass slides, glass beads, centrifuge tubes, nitrocellulose membranes, nylon membranes, and derivatized nylon membranes, and also particles, such as magnetic particles, agarose, SEPHADEX™, and the like.

For many types of assays, such as sandwich assays and the like, it is desirable to attach to the solid support a capture reagent that can specifically bind to a target analyte of

interest. Capture reagents can be attached to the solid support either covalently or non-covalently, can be pre-immobilized on the solid support prior to contacting the sample, or can become immobilized during or after the course of the binding to the target analyte. For example, the capture reagent can include a tag that mediates binding of the capture reagent to the solid support. The tag can be any of a variety of components. In general, a molecule which binds the tag (a tag binder) is fixed to a solid support, and the tagged capture reagent is attached to the solid support by interaction of the tag and the tag binder. A number of tags and tag binders can be used, based upon known molecular interactions well described in the literature. For example, where a tag has a natural binder, for example, biotin, protein A, or protein G, it can be used in conjunction with appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an immunoglobulin, etc.). Antibodies to molecules with natural binders such as biotin are also widely available and appropriate tag binders; *see, SIGMA Immunochemicals 1998 catalogue SIGMA, St. Louis MO).*

Similarly, any haptic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. *See, Id.* Indeed, the antibody can be either the tag or the tag binder, or antibodies can be used as both tags and tag binders. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody which recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs. For example, agonists and antagonists of cell membrane receptors (*e.g.*, cell receptor-ligand interactions such as *e.g.*, transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherin family, the integrin family, the selectin family, and the like; *see, e.g.*, Pigott and Power (1993) *The Adhesion Molecule FactsBook*, Academic Press New York, and Hulme (ed) *Receptor Ligand Interactions A Practical Approach*, Rickwood and Hames (series editors) Hulme (ed) IRL Press at Oxford Press NY). Similarly, toxins and venoms, viral epitopes, hormones (*e.g.*, opiates, steroids, etc.), intracellular receptors (*e.g.* which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and

cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

Synthetic polymers such as heteropolymers in which a known drug is covalently bound to any of the above, such as polyurethanes, polyesters, polycarbonates, 5 polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

Specific tag-tag binder interactions occur when the tag and tag binder bind with a K_D of at least about 0.01 μM , preferably at least about 0.001 μM or better, and most typically and preferably, 0.0001 μM or better, under standard assay conditions.

Methods for the attachment of tags to molecules that can bind to a particular target analyte are known to those of skill in the art. For example, where the binding moiety (or "recognition moiety") is a polypeptide, a preferred embodiment of preparing a capture reagent involves producing a fusion protein by recombinant methods. For example, a polynucleotide encoding the recognition moiety is operably linked to a polynucleotide that encodes an epitope for which convenient means of detection exist. The polynucleotide encoding the epitope is preferably placed at a location relative to the recognition moiety coding sequence that does not disrupt the ability of the fusion protein to bind to the target analyte. Methods for constructing and expressing genes that encode fusion proteins are well known to those of skill in the art. Examples of techniques and instructions sufficient to direct persons of skill through these exercises are found in, for example, Sambrook, Ausubel, and Berger, all *supra*.

Examples of suitable epitopes include the *myc* and V-5 reporter genes; expression vectors useful for recombinant production of capture reagents having these epitopes are commercially available (e.g., Invitrogen (Carlsbad CA) vectors pcDNA3.1/Myc-His and pcDNA3.1/V5-His are suitable for expression in mammalian cells). Additional expression vectors for suitable fusion proteins, and corresponding detection systems are known to those of skill in the art, and several are commercially available (e.g., FLAGTM (Kodak, Rochester NY)). Another example of a suitable tag is a polyhistidine sequence, which is capable of binding to metal chelate affinity ligands. Typically, six

adjacent histidines are used, although one can use more or less than six. Suitable metal chelate affinity ligands that can serve as the binding moiety for a polyhistidine tag include nitrilo-tri-acetic acid (NTA) (Hochuli, E. (1990) "Purification of recombinant proteins with metal chelating adsorbents" *In Genetic Engineering: Principles and Methods*, J.K. Setlow, 5 Ed., Plenum Press, NY; commercially available from Qiagen (Santa Clarita, CA)).

Other haptens that are suitable for use as tags are known to those of skill in the art and are described, for example, in the *Handbook of Fluorescent Probes and Research Chemicals* (6th Ed., Molecular Probes, Inc., Eugene OR). For example, dinitrophenol (DNP), digoxigenin, barbiturates (*see, e.g.*, US Patent No. 5,414,085), and several types of fluorophores are useful as haptens, as are derivatives of these compounds. Kits are commercially available for linking haptens and other moieties to proteins and other molecules. For example, the hapten includes a thiol, a heterobifunctional linker such as SMCC can be used to attach the tag to lysine residues present on the capture reagent.

Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivitized or functionalized by exposing all or a portion of the substrate to a chemical reagent which fixes a chemical group to the surface which is reactive with a portion of the tag binder. For example, groups that are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer arrays is well described in the literature. *See, e.g.*, Merrifield (1963) *J. Am. Chem. Soc.* 85: 2149-2154 (describing solid phase synthesis of, *e.g.*, peptides); Geysen *et al.* (1987) *J. Immun. Meth.* 102: 259-274 (describing synthesis of solid phase components on pins). *See,* Frank and Doring (1988) *Tetrahedron* 44: 6031-6040 (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al.* (1991) *Science* 251: 767- 777; Sheldon *et al.* (1993) *Clinical Chemistry* 39(4): 718-719 and Kozal *et al.* (1996) *Nature Medicine* 2(7): 25 753-759 (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

Where the capture reagent is a DNA or RNA nucleic acid, attachment to any of the various suitable solid supports is likewise performed using available techniques. For

example, one can attach to the nucleic acid a tag which has an affinity to a derivatized solid support. Suitable tags and corresponding tag binders are discussed above. In one embodiment, linkers are added to the nucleic acid and attachment to the tag is performed through the linker. Common linkers include polypeptide sequences, such as poly-gly sequences of between about 5 and 200 amino acids. In some embodiments, proline residues are incorporated into the linker to prevent the formation of significant secondary structural elements by the linker. Such flexible linkers are known to persons of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc., Huntsville, Alabama. These linkers optionally have amide linkages, sulphydryl linkages, or heterofunctional linkages. Solid supports which have suitable derivatives for immobilization of DNA are commercially available (e.g., DNA-Bind plates, Costar).

Similarly, the tagged nucleic acid can be directly attached to a solid substrate in the assays of the invention. In this embodiment, the terminal end of the nucleic acid is, itself, the molecular tag. In this embodiment, tagged nucleic acids are fixed to or synthesized on a solid support. For example, using chip masking technologies and photoprotective chemistry it is possible to generate arrays of nucleic acid probes. These arrays, which are known, e.g., as "DNA chips," can include millions of nucleic acid regions on a substrate having an area of about 1 cm² to several cm², thereby incorporating sets of from a few to millions of tagged nucleic acids. See, e.g., Fodor *et al.* (1991) *Science* 251: 767- 777; Sheldon *et al.* (1993) *Clinical Chemistry* 39(4): 718-719, and Kozal *et al.* (1996) *Nature Medicine* 2(7): 753-759.

The capture reagent can become attached to the non-stick coated solid support before, after, or simultaneously with the binding of the target analyte to the capture reagent. For example, one can add the capture reagent to the sample prior to contacting the sample with the solid support. Alternatively, one can attach the capture reagent to the solid support prior to contacting the sample. In any case, once the solid support, capture reagent, and target analyte are incubated for sufficient time for the target analyte to become immobilized on the solid support, the presence or absence of the target analyte is then detected.

Signal Generation and Detection

Many methods of detecting a target analyte are known to those of skill in the art. In a sandwich assay, for example, an immobilized target analyte can be detected through

use of a signal reagent (which term includes "signal probe" as used herein). The signal reagent typically includes a recognition moiety that can specifically bind to the particular target analyte, and also a label. Recognition moieties can be prepared as described above for capture reagents.

5 The labels used in the assays of the present invention can be primary labels (where the label comprises an element that is detected directly or that produces a directly detectable element) or secondary labels (where the detected (primary) label is attached to a moiety that binds to the secondary label, *e.g.*, as is common in immunological labeling). An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden (1997) *Introduction to Immunocytochemistry*, 2nd ed., Springer Verlag, NY and in Haugland (1996) *Handbook of Fluorescent Probes and Research Chemicals*, a combined handbook and catalogue Published by Molecular Probes, Inc., Eugene, OR. Primary and secondary labels can include undetected elements as well as detected elements. Useful primary and secondary labels in the present invention can include spectral labels such as fluorescent dyes (*e.g.*, fluorescein and derivatives such as fluorescein isothiocyanate (FITC) and Oregon Green™, rhodamine and derivatives (*e.g.*, Texas red, tetrarhodamine isothiocyanate (TRITC), etc.), digoxigenin, biotin, phycoerythrin, AMCA, CyDyes™, and the like), radiolabels (*e.g.*, ^3H , ^{125}I , ^{35}S , ^{14}C , ^{32}P , ^{33}P , etc.), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase *etc.*), spectral colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.* polystyrene, polypropylene, latex, etc.) beads. The label can be coupled directly or indirectly to a signal reagent according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

20 Preferred labels include those that use: 1) chemiluminescence (using horseradish peroxidase and/or alkaline phosphatase with substrates that produce photons as breakdown products as described above) with kits being available, *e.g.*, from Molecular Probes, Amersham, Boehringer-Mannheim, and Life Technologies/ Gibco BRL; 2) color production (using both horseradish peroxidase and/or alkaline phosphatase with substrates that produce a colored precipitate [kits available from Life Technologies/Gibco BRL, and Boehringer-Mannheim]); 3) hemifluorescence using, *e.g.*, alkaline phosphatase and the

substrate AttoPhos (Amersham) or other substrates that produce fluorescent products, 4) fluorescence (*e.g.*, using Cy-5 [Amersham]), fluorescein, and other fluorescent tags); 5) radioactivity. Other methods for labeling and detection will be readily apparent to one skilled in the art.

One example of a labeling strategy that is particularly susceptible to the adverse effects that can be avoided by using the methods of the invention uses an enzyme as the label. These methods use an antibody (for a sandwich immunoassay) or a complementary oligonucleotide (for a nucleic acid sandwich hybridization assay) that is linked to an enzyme (typically by recombinant or covalent chemical bonding). The signal reagent is detected when the enzyme is placed in contact with its substrate, producing a detectable product.

Preferred enzymes that can be conjugated to signal reagents of the invention include, *e.g.*, β -galactosidase, luciferase, horse radish peroxidase, lysozyme, glycose-6-phosphate dehydrogenase, lactate dehydrogenase, urease, cholesterol oxidase, and alkaline phosphatase. A chemiluminescent substrate for luciferase is luciferin. One example of a chemiluminescent substrate for β -galactosidase is 4-methylumbelliferyl- β -D-galactoside. Embodiments of alkaline phosphatase substrates include p-nitrophenyl phosphate (pNPP); 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) and fast red/naphthol AS-TR phosphate; and 4-methoxy-4-(3-phosphonophenyl) spiro[1,2-dioxetane-3,2'-adamantane]. Embodiments of horseradish peroxidase substrates include 2,2'azino-bis(3-ethylbenzthiazoline-6 sulfonic acid) (ABTS), 5-aminosalicylic acid (5AS), o-dianisidine, and o-phenylenediamine (OPD); and 3,3,5,5'-tetramethylbenzidine (TMB), 3,3'diaminobenzidine (DAB), 3-amino-9-ethylcarbazole (AEC), and 4-chloro-1-naphthol (4C1N). Other suitable substrates are known to those skilled in the art. The enzyme-substrate reaction and product detection are performed according to standard procedures known to those skilled in the art and kits for performing enzyme immunoassays are available as described above.

In general, a detector which monitors a particular probe or probe combination is used to detect the signal reagent label. Typical detectors include spectrophotometers, phototubes and photodiodes, microscopes, scintillation counters, cameras, film and the like, as well as combinations thereof. Examples of suitable detectors are widely available from a variety of commercial sources known to persons of skill. Commonly, an optical image of a substrate comprising bound labeling moieties is digitized for subsequent computer analysis.

EXAMPLES

The following examples are offered to illustrate, but not to limit the present invention.

Example 1

This Example demonstrates that SDS and n-lauryl sarcosine can be carried from a sample solution to a signal detection solution by a solid support, even when the support is washed prior to being placed in the signal detection solution. This carryover caused aberrant results such as substrate precipitation and dropout.

The materials used in this example were as follows: TMB (3,3,5,5'-tetramethylbenzidine; Research Diagnostics Inc., Flanders NJ), Assay Wash (0.1 M NaHPO₄, 0.5% Tween-20), a target nucleotide, a capture oligonucleotide that has a 21 nucleotide region that is complementary to a portion of the target nucleotide, and a signal nucleotide that has a 24 nucleotide region that is complementary to a different portion of the target nucleotide, Anti-Fluor POD (peroxidase; diluted 1:1000 (Boehringer Mannheim #1426346)), sample solution (5M GuSCN, 0.08 M Tris, 0.0167M EDTA, sample solution base (0.08M Tris, 0.0167M EDTA, pH 8 (contains no GuSCN)), lysis solution (0.0908M Tris, 0.0099M EDTA, either 0.5% or 0.25% SDS, 5% n-lauroyl sarcosine, and 0.1% Proclin), signal solution base (0.05M Tris, 0.01M EDTA, 0.5% Tween-20).

The prongs used in the automated analyzer were coated with streptavidin as follows. First, the prongs were dipped in 95% ethanol for about 2 minutes, after which they were allowed to dry for about 10 minutes. The prongs were then incubated for 1 hour at room temperature in 150 µl of 10µg/ml streptavidin. Finally, they were washed twice with 200 µl of assay wash solution.

The assays were carried out in a Saigene automated analyzer, which is designed to carry a solid-phase plastic prong strip through the steps of a sandwich hybridization assay. The reagents for the assay are dispensed in a 96 well microwell plate. The processor is programmed with instructions contained on a program card (2MB8); the program instructs the processor as to the dwell time in each well. These times are outlined in Table 1. Eight rows of wells were used, with each row corresponding to one step of the analyzing steps. The analyzer was programmed to place the solid support prongs in the capture reagent (contains 200 ng/ml of the capture oligonucleotide in assay wash solution)

for ten minutes, after which the prongs were placed in the sample solution (contains the target oligonucleotide in 3M GuSCN (which was diluted in a ratio of 60:40 from a 5M solution)) for ten minutes. Next, the prongs were moved to a row of wells that contained the signal reagent (signal oligonucleotide at 300 ng/ml in signal solution; 2 M GuSCN) for five minutes. The prongs were then placed in a wash solution for 1 minute, after which they were placed in the conjugate solution (anti-fluorescein HRP conjugate) for five minutes. Two more washes followed (1 minute followed by two minutes, after which the prongs were placed in the signal development solution, which contained TMB, for eight minutes.

D 10 20 30 40 50 60 70 80 90 100

Table 1: Basic Plate Setup

WELL	STEP	TIME (minutes)	REAGENT
8	8	8	Substrate
7	7	2	Wash
6	6	1	Wash
5	5	5	Conjugate
4	4	1	Wash
3	3	5	Signal
2	1	10	Capture
1	2	10	Sample

Following the reaction, the substrate-containing wells were examined for the presence of precipitated materials. The experiment in which the lysis solution contained 0.25% SDS resulted in precipitation in 3 out of 12 wells. The experiment in which the lysis solution contained 0.5% SDS showed precipitation in 9 out of 12 wells. Therefore, 15 carryover of SDS and n-lauroyl sarcosine from a sample solution to the enzyme substrate solution can cause precipitation of the substrate.

Example 2

This Example demonstrates that coating the prongs with a non-stick agent such as GelSlick™ can reduce or eliminate carryover.

The materials used in this experiment were as described in Example 1, except 5 that the lysis solution contained 0.25% SDS instead of 0.5%.

The prongs in the control group were coated only with streptavidin, whereas in the experimental group, the prongs were coated with GelSlick™ first, then with streptavidin. For the control group, the prongs were dipped in 95% ethanol for about 2 minutes. After allowing the prongs to dry for about 10 minutes, the prongs were further incubated for 1 hour at room temperature in 100µl of 10µg/ml streptavidin. Finally, the prongs were washed twice with 200µl of assay wash solution.

For the experimental group, the prongs were dipped in 95% ethanol for about 2 minutes and allowed to dry for about 10 minutes. Meanwhile, GelSlick™ was diluted in water to 2%, and 75ml of the diluted GelSlick™ was added to a small tray, such as a lid from a pipette tip box. The prongs were placed upside down in the tray, making sure that the solution surface did not touch the ball end of the prongs. This way, the prong stems were coated with GelSlick™, but not the ball ends. (Later experiments demonstrated that the non-stick coating does not interfere with the streptavidin coating of the prong; therefore, in preferred embodiments the entire prong strip is coated with nonstick reagent after the prongs 20 are coated with streptavidin.) The prongs were then removed and allowed to dry, after which the prongs were incubated further in 100µl of 10 µg/ml streptavidin for one hour at room temperature, and then washed twice with 200 µl assay wash.

For each of the two groups, three different amounts of streptavidin were used: 75, 100, and 150 µl of 10 µg/ml streptavidin.

A standard diagnostic assay was performed using both sets of prongs. The 25 standard diagnostic assay was run with both the experimental group and the control group. See Table 1 for the basic plate set-up of the assay. Reagents were as described in Example 1.

Table 2: Comparison of Coated and Uncoated Prongs

coat vol:	Standard Prongs			GelSlick™-Coated Prongs		
	75 µl	100µl	150 µl	75 µl	100 µl	150 µl
1	0.662	0.672	0.631	2.719	4.111	1.946
2	3.922	4.018	>4.2	3.697	3.977	>4.2
3	3.490	3.757	1.490	3.178	3.691	>4.2
4	3.696	4.007	3.823	3.274	4.055	3.823

coat vol:	Standard Prongs			GelSlick™-Coated Prongs		
	75 µl	100µl	150 µl	75 µl	100 µl	150 µl
5	3.829	3.856	>4.2	3.476	3.939	>4.2
6	2.408	4.029	3.923	3.257	3.942	>4.2
7	0.394	4.067	4.176	3.395	3.067	>4.2
8	3.941	3.748	4.980	3.588	4.010	>4.2
9	4.100	4.044	3.894	3.512	4.098	>4.2
10	1.853	3.590	0.040	3.874	>4.2	>4.2
11	0.708	0.537	3.956	4.024	>4.2	>4.2
12	3.907	0.598	0.474	4.128	4.157	>4.2
AVE	2.743	3.077	2.829	3.510	3.906	2.885
SD	1.4635	1.4996	1.7246	0.3919	0.3213	1.3272
CV	53%	49%	61%	11%	8%	46%

Average w/ off scale
values calculated at
4.2:

3.057

3.955

3.980

The shaded entries indicate drop-outs and/or samples in which precipitation occurred, and samples that for which the absorbance was off-scale are shown as >4.2.

As shown in Table 2, the overall signal strength increased as the amount of coating solution increased. Prongs coated with GelSlick™ had overall about 1 OD higher signal at each coating volume than those not coated with GelSlick™ (Figure 1). Thus, the results from the two groups indicated that coating the prong with GelSlick™ increased the sensitivity of the assay.

Example 3

In the previous Example, the prongs were coated with a non-stick material prior to attachment of the capture reagent. This Example demonstrates that one can coat the prongs with a non-stick material after they have been coated with a capture reagent, in this case, streptavidin.

Materials used in this experiment were as described in Example 1, except that the lysis solution contained 0.25% SDS, and the signal reagent was biotin-HRP at a concentration of 12.5 ng/ml.

The prongs were prepared as follows. First, the prongs were dipped in 95% ethanol for about 2 minutes and allowed to dry for about 10 minutes. Next, they were incubated in 100µl of 10 µg/ml streptavidin at room temperature for 1 hour. Then, prongs were washed twice with 200µl of the assay wash solution. The prongs were then coated with

GelSlick™ by dipping them in a 2% GelSlick™ (in water, sonicated to an emulsion) for about 2 minutes, and allowed to dry.

Two groups of experiments were run. As a control, a "mock" assay was run without the capture, signal and linker reagents. The second group was the experimental group which included each of these reagents. Both groups were run in duplicate using different hybridization and wash volumes. In one set of assays, 200µl of each assay reagent were placed in the wells. In another set, either the volume of the hybridization solution (150µl) used in the sample and signal wells (samples 1 and 3), or the volume of the assay wash solution (250µl; samples 4, 5 and 7) were varied. The basic plate setup is shown in Table 3.

Table 3: Basic Plate Setup

WELL	STEP	TIME (minutes)	REAGENT
8	8	8	Substrate
7	7	1	Wash
6	6	5	Conjugate
5	5	1	Wash
4	4	1	Wash
3	3	5	Signal
2	1	10	Capture
1	2	10	Sample

The results from the experiment are shown in Table 4 and in Figures 2A and 15 2B. Reproducible results were obtained using this procedure, as only two dropouts occurred in the experiment, one in each of the repetition with adjusted volumes. Therefore, subsequent coating of the prongs with the non-stick material did not interfere with the ability of the streptavidin to bind to the biotin.

Table 4

	Run #1		Run #2	
	STD-200	150/250	STD-200	150/250
1	1.901	1.329	1.732	1.369
2	2.050	1.949	1.956	1.119
3	1.824	2.027	1.591	0.866
4	1.850	2.045	1.720	1.188
5	1.792	2.046	1.537	1.485
6	1.370	2.090	1.653	1.213
7	2.032	1.864	1.542	1.559
8	2.013	2.024	1.690	1.638
9	1.997	2.080	1.691	1.591
10	1.643	1.915	1.787	1.696
11	1.978	1.964	1.952	1.524
12	1.656	0.687	1.656	1.925
AVE	1.842	1.835	1.725	1.431
SD	0.2033	0.4153	0.1410	0.2920
CV	11%	23%	8%	20%

Example 4

This Example evaluates the effect of coating the prongs with 0.1% silane vs.

5 2% GelSlick™ on the reduction of substrate precipitation. In this Example, prongs were coated with streptavidin before they were coated with either silane or GelSlick™.

Materials used in this experiment were as described in Example 3, except that the Sample solution, Sample solution base, Signal solution and signal solution base contained no formamide.

10 Two groups of experiments were performed: in the Silane group, prongs were coated with diluted silane, and in the GelSlick™ group, the prongs were coated with GelSlick™.

The prongs were coated as follows. First, the prong reservoir was filled with 27 total ml of ethanol: 2 ml into a separate well for each prong, and the remaining 25 ml into a common reservoir. Then the filled prong-cassette was inserted into the reservoir and

allowed to soak for 2 minutes. The prong-cassette was removed and placed in a dry, empty reservoir and the prongs were allowed to fully dry for about 20 minutes. Wells of a Falcon flexible assay plate (#912) were filled with 100 µl of streptavidin at 10 µg/ml (diluted in coating buffer). The prongs were coated by snapping the filled prong-cassette into the assay plate containing the streptavidin and incubating for one hour at room temperature. The prong-cassette was removed, and the prongs were washed twice by emptying the wells of the coating solution, filling the wells with 200 µl of assay wash, and then inserting the prongs. The prongs were then removed, and wells of used wash were emptied. The wash was repeated once. The prongs were then allowed to air dry for 15 minutes.

While the prongs were drying, the secondary coating solution was made up. For the GelSlick™ group, GelSlick™ was diluted to 2% with water (4ml GelSlick™ + 196ml water). For the silane group, silane was diluted to 0.1% with water. The solution was vortexed to begin mixing. After vortexing, the solution was set in a sonicator for 10 minutes (power set at 50%, degas set at 100%).

Sonicated GelSlick™ solution was added to prongs in a small tray. Mixing was performed on an orbital shaker for 5 minutes, ensuring that the fluid level is above the ball of the prongs. The prongs were then dried in a desiccator for 30 minutes. Vacuum was set by pulling full draw on the container for 1.5 minutes.

The basic plate setup for this assay is shown in Table 5.

20

Table 5: Basic Plate Setup

WELL	STEP	TIME (minutes)	REAGENT
8	8	8	Substrate
7	7	1	Wash
6	6	5	Conjugate
5	5	1	Wash
4	4	1	Wash
3	3	5	Signal

WELL	STEP	TIME (minutes)	REAGENT
2	1	10	Capture
1	2	10	Sample

The results of this experiment, which are shown in Table 6, demonstrate that 0.1% silane and 2% GelSlick™ were similarly effective in reducing substrate precipitation.

Table 6

	0.1% Silane	2% GelSlick™
1	2.023	1.848
2	2.124	1.369
3	2.020	1.880
4	2.038	1.481
5	1.978	1.652
6	1.696	1.821
7	1.946	1.737
8	1.721	1.847
9	1.726	2.015
10	2.007	1.881
11	2.017	2.119
12	0.253	2.144
AVE	1.796	1.816
SD	0.5062	0.2325
CV	28%	13%
	1.936	
	0.1489	
	8%	

Example 5

This Example provides a protocol for coating a solid support with a non-stick coating.

The materials used in this experiment were as described in Example 4. The test solution was prepared by mixing Sample solution and Lysis solution in a ratio of 60:40, together with 5 µg/ml polyadenylic acid. 5MB9 program card was used in the analyzer, as were prongs obtained from Becton and Dickinson (Sparks, MD).

A. Prong preparation

The prong reservoir was filled with 27 ml 95% ethanol: 2 ml into the separate well and the remaining 25ml into the common reservoir. The filled prong-cassette was inserted into the reservoir and allowed to soak for 2 minutes, after which the prong-cassette was removed and placed in a dry, empty reservoir. The prongs were allowed to fully dry for about 20 minutes.

Wells of a Falcon flexible assay plate (#912) were filled with 100 µl of streptavidin at 10 µg/ml (diluted in coating buffer). Prongs were coated by snapping the filled prong-cassette into the assay plate containing the streptavidin and incubating for one hour at room temperature. The prong-cassette was then removed, and the prongs were washed twice by emptying the wells of coating solution, filling them with 200 µl of assay wash, and then inserting the prongs. Again, the prongs were removed and wells of used wash were emptied. This procedure was repeated once. The prongs were then air-dried for 15 minutes.

While the prongs were drying, a secondary coating solution was made up by diluting GelSlick™ to 2% with water (4 ml GelSlick™ + 196 ml water). The coating solution was mixed by vortexing, after which the solution was set in a sonicator for 10 minutes (power set at 50%; degas set at 50%). The sonicated GelSlick™ solution was added to the prongs in a small tray, and then mixed on an orbital shaker for five minutes.

The filled prong-cassette was then submerged in 75% ethanol for about one minute. Then the prongs were removed and excess ethanol was shaken off of the prong surface. Afterwards, the prongs were dried in a desiccator for 30 minutes. Vacuum was set by pulling full draw on the container for 1.5 minutes.

B. Assay

The assay was performed with Signal solution at 1 M and sample solution at 2M (diluted 60:40 from a 3.33M solution). This run included 5 µg/ml of polyadenylic acid in the sample solution. To facilitate the comparison of this run to previous experiments, two strips of prongs were run in which the sample solution did not contain any polyadenylic acid. As a further control, a standard "mock" assay was run without capture, signal or linker reagents.

The Basic plate set-up is similar to those in the previous examples (Table 7).

Table 7: Basic Plate Setup

WELL	STEP	TIME (minutes)	REAGENT
8	8	8	Substrate
7	7	2	Wash
6	6	5	Conjugate
5	5	2	Wash
4	4	2	Wash
3	3	5	Signal
2	1	10	Capture
1	2	10	Sample

The results of this experiment are shown in Table 8. The data shown in “Control #1” and “Control #2” columns, in which the samples lacked polyA, demonstrate that this protocol provided results that compare well with those of earlier experiments that did not contain polyA. The average absorbance obtained with polyA-containing samples was slightly lower than that obtained with no polyA, and the standard deviation was slightly higher. However, this slight increase in variability is almost entirely due to a “trough effect” that occurred in Runs #2 and #3 (underlined values).

Table 8

2' Coated Prongs (020597 ptcl) Vinyl Plate								
	Control #1	Control #2	#1	#2	#3	#4	#5	#6
1	2.118	2.247	2.211	2.038	2.199	2.305	2.077	1.935
2	2.106	2.057	2.219	1.995	2.097	2.117	2.214	2.108
3	2.181	2.145	2.066	1.984	2.000	2.001	2.023	2.039
4	2.139	2.003	1.958	1.744	1.949	2.012	2.080	2.054
5	1.990	2.134	2.018	<u>1.788</u>	1.828	1.930	1.980	2.074
6	2.159	2.067	2.035	<u>1.551</u>	<u>1.741</u>	1.875	1.950	2.166
7	2.125	1.974	2.033	<u>1.539</u>	<u>1.642</u>	1.917	1.979	1.918
8	2.174	2.072	1.915	<u>1.635</u>	<u>1.684</u>	1.937	2.032	2.002
9	2.122	2.001	1.943	<u>1.496</u>	<u>1.732</u>	1.989	2.002	2.124
10	2.270	1.917	2.028	<u>1.690</u>	1.890	2.096	1.970	2.102
11	2.133	1.923	2.029	1.990	2.011	2.205	1.967	2.035
12	2.025	2.147	2.096	2.179	2.265	2.363	2.073	1.875
AVE	2.129	2.057	2.046	1.802	1.920	2.062	2.029	2.036
SD	0.0717	0.0995	0.0940	0.2283	0.2030	0.1580	0.0740	0.0890
CV	3%	5%	5%	13%	11%	8%	4%	4%
tAV	2.093		1.983					
E	0.0922		0.1747					
tSD	4%		9%					
tCV								

Example 6

This example illustrates the use of a solid support coated with a silanizing agent in reducing detergent-caused substrate precipitation and enzyme inactivation in immunoassays.

Immunoassays are often performed using as samples cell lysates or other relatively impure mixtures that contain a target molecule of interest. These mixtures can contain detergents and other agents that can interfere with the immunoassay. The use of a

silanizing agent to coat prongs as described in this Example can prevent such contaminants from having an adverse effect on the assay.

The prongs are coated with a silanizing agent such as Gel Slick™ as described in the previous Examples. The coated prongs are then coated with protein A, which can bind IgG immunoglobulins.

To perform the immunoassay, the prongs are first placed into a solution that contains an IgG immunoglobulin that is specific for the target molecule. This "capture reagent" is bound by the IgG constant region, leaving the variable regions of the immunoglobulin available to bind the target molecule.

The prongs are then placed in wells that contain a sample, such as a cell lysate. After incubation in the sample for a sufficient time for the target molecules, if present, to bind to the protein A, the prongs are moved to wells that contain a signal reagent such as an antibody that can bind to the target molecule. The antibody is labeled with, for example, an enzyme. The prongs are then washed to remove any unbound materials, including detergents or other agents present in the sample that could interfere with the immunoassay. After washing, the prongs are placed into a substrate solution that includes reagents (e.g., substrates for the enzyme) that exhibit a detectable change when the signal reagent is present.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.